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A Phase I Study of Adenovirus-Mediated Wild-Type *p53* Gene Transfer in Patients with Advanced Non-Small Cell Lung Cancer

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ABSTRACT

Mutations of the tumor suppressor gene *p53* are the most common genetic alterations observed in human cancer. Loss of wild-type *p53* function impairs cell cycle arrest as well as repair mechanisms involved in response to DNA damage. Further, apoptotic pathways as induced by radio- or chemotherapy are also abrogated. Gene transfer of wild-type *p53* was shown to reverse these deficiencies and to induce apoptosis *in vitro* and in preclinical *in vivo* tumor models. A phase I dose escalation study of a single intratumoral injection of a replication-defective adenoviral expression vector encoding wild-type *p53* was carried out in patients with incurable non-small cell lung cancer. All patients enrolled had p53 protein overexpression as a marker of mutant p53 status in pretreatment tumor biopsies. Treatment was performed either by bronchoscopic intratumoral injection or by CT-guided percutaneous intratumoral injection of the vector solution. Fifteen patients were enrolled in two centers, and were treated at four different dose levels ranging from 10^7 to 10^{10} PFU (7.5×10^9 to 7.5×10^{12} particles). No clinically significant toxicity was observed. Successful transfer of wild-type *p53* was achieved only with higher vector doses. Vector-specific wild-type *p53* RNA sequences could be demonstrated in posttreatment biopsies of six patients. Transient local disease control by a single intratumoral injection of the vector solution was observed in four of those six successfully transduced patients. There was no evidence of clinical responses at untreated tumor sites. Wild-type *p53* gene therapy by intratumoral injection of a replication-defective adenoviral expression vector is safe, feasible, and biologically effective in patients with advanced non-small cell lung cancer.

OVERVIEW SUMMARY

Gene transfer of the human wild-type (wt) *p53* tumor suppressor gene into *p53* mutant tumors has been shown to have antiproliferative and apoptotic effects *in vitro* and *in vivo*. Schuler *et al.* report on a phase I clinical study of local wt *p53* gene therapy in patients with advanced non-small cell lung cancer. A recombinant replication-deficient adenovirus carrying the complete human wt *p53* cDNA under the control of the CMV immediate/early gene promoter was devised as expression vector. Gene transfer was achieved at higher virus doses without occurrence of any relevant treatment-related toxicity. In addition, clinical effects suggestive of a moderate local antiproliferative activity were observed in some patients.

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INTRODUCTION

Mutations of the tumor suppressor gene *p53* are detected in more than 50% of human cancers. Missense mutations within the sequence coding for the DNA-binding domain of the *p53* gene product are the most common alterations observed (Hollstein *et al.*, 1994). These mutations result in synthesis of a functionally inactive protein, which accumulates intracellularly at high levels (Bartek *et al.*, 1991). Less frequently, *p53* deletions or chain-termination mutations are detected. Loss of functional p53 results in impaired cell cycle control and repair mechanisms in response to DNA damage (Levine, 1997). Patients suffering from the Li-Fraumeni syndrome are, as a result of germline mutation of the *p53* gene, prone to early cancer development, as are mice with a homozygous deletion of *p53* (Donehower *et al.*, 1992). Moreover, *p53* mutations may abrogate apoptosis as induced by several cytotoxic agents or radiation (Lowe *et al.*, 1993a,b). In light of this preclinical evidence, restoration of functional p53 represents an attractive target for somatic gene therapy in cancer. Results of a pilot study of nine patients support this view (Roth *et al.*, 1996).

Non-small cell lung cancer (NSCLC) accounts for 75 to 80% of all lung cancers, and is still a leading cause of death from malignancy (Boring *et al.*, 1994). At primary diagnosis, most patients present with nonresectable disease. Despite intensive chemo- and radiotherapy, 5-year survival of stage III patients does not exceed 15% (Dillman *et al.*, 1996; Pritchard and Anthony, 1996). In stage IV NSCLC, chemotherapy never is curative and prolongs median survival for less than 4 months as compared with best supportive care (Cellerino *et al.*, 1991; Marino *et al.*, 1994). Thus, new treatments for advanced NSCLC are clearly needed.

Structural alterations of the *p53* gene are detected in about 45 to 50% of tumors of NSCLC patients (Takahashi 1989), and are associated with an adverse prognosis (Quinlan *et al.*, 1992; Nishio *et al.*, 1996; Ohsaki *et al.*, 1996). Further, mutant *p53* status might also contribute to the low response rates to chemo- and radiotherapy observed in NSCLC. The present phase I study was undertaken to assess safety, feasibility, and biological activity in terms of transgene expression status of local adenovirus-mediated *p53* gene therapy in patients with advanced NSCLC exhibiting *p53* mutations.

PATIENTS AND METHODS

Patients

All patients enrolled in the study had histologically confirmed stage III B or IV NSCLC with evidence of *p53* gene mutation in the tumor tissue. Immunohistochemical detection of intratumoral p53 protein accumulation by monoclonal antibodies PAb 1801 and/or PAb 240 (Pharmingen, San Diego, CA) served as surrogate marker of mutant *p53* status (Bartek *et al.*, 1991). Staining of paraffin-embedded tissue was performed according to standard methods. Sections with definitive nuclear reactivity of >30% of cells were scored 3+; sections with a reactivity of 25 to 50% of cells were scored 2+; and sections with a reactivity of 11 to 24% of cells were scored 1+. Sections with 10% or fewer reactive cells were scored as negative. Additional inclusion criteria were as follows: age, 18 to 75 years; a Karnofsky performance score of at least 70%; an absence of clinically relevant hematologic, hepatic, or renal insufficiency or electrolyte imbalances; and pulmonary function adequate to perform all treatment procedures safely. A treatment-free interval of at least 4 weeks in duration had to have elapsed before enrollment in the study. Pregnant or lactating women, fertile women not practicing medically acceptable contraception for at least 6 months following study treatment, patients with uncontrolled serious infections, human immunodeficiency virus (HIV) positivity, or patients receiving systemic immunosuppressive or corticosteroid treatment within the last 3 months before entry into the study were excluded. Acute adenoviral infection was ruled out prior to therapy. All patients gave written informed consent.

Methods

Study design. This was a bicentric, open, phase I dose escalation study. Three patients were treated at each dose level, and dose escalation was continued until two consecutive dose levels confirmed biological activity of the treatment as defined by demonstration of intratumoral transgene expression, or until significant dose-limiting toxicity was encountered. Each patient was monitored weekly for 28 days, with day 1 being the date of treatment. On completion of the observation period all patients were monitored at the study centers at regular intervals. The protocol was approved by the local ethics committees, and by the national regulatory offices (Germany: Kommission Somatische Gentherapie der Bundesärztekammer, and Zentralkommission Biologische Sicherheit; Switzerland: Schweizerische Kommission für Biologische Sicherheit, and Interkantonale Arzneimittelstelle). The study was performed according to the Declaration of Helsinki and according to the principles of good clinical practice.

Study end points. Primary objectives of the study were to determine safety, feasibility, and biological activity of a single intratumoral injection of SCH 58500, as defined by reverse transcription and polymerase chain reaction (RT-PCR) detection of vector-specific wild-type (wt) *p53* RNA sequences in post-treatment tumor biopsies. The secondary objective was to assess clinical evidence of antitumoral efficacy of intratumoral SCH 58500 injection in patients with NSCLC.

Staging procedures. At baseline and on day 28 all patients underwent computed tomography (CT) scans of the chest for bidimensional measurement of the treated tumor lesion and all other tumor manifestations. If clinically indicated, CT or ultrasound scans of the abdomen and bone scans were performed.

Treatment. SCH 58500 (rAd/p53) is an aqueous solution of a replication-defective recombinant adenovirus type 5 containing the complete human wt *p53* cDNA under the control of the human cytomegalovirus (CMV) immediate-early gene promotor (Wills *et al.*, 1994; Harris *et al.*, 1996). SCH 58500 was manufactured in CGMP compliance in validated plant facilities under strict environmental monitoring and control conditions by Schering-Plough Werthenstein Chemie AG (Schachen, Switzerland). In brief, the vector was propagated in 293 cells

in a bioreactor. The cells were grown in liquid medium on the surface of microcarriers. As a part of routine testing, each fermentation batch was tested for sterility, mycoplasma, adventitious viruses, and adeno-associated virus at the unprocessed bulk stage. The SCH 58500 product was purified by chromatography (Huyghe *et al.*, 1995) and filtration, and was supplied in vial strengths of 1×10^8 plaque-forming units per milliliter (PFU/ml) and 1×10^9 PFU/ml (7.5×10^{10} and 7.5×10^{11} particles/ml). Patients were treated with a single intratumoral injection of four different dose levels of SCH 58500 (absolute 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} PFU). In dose levels 1, 2, and 3 (endobronchial) a volume of 1 ml of SCH 58500 was injected intratumorally at bronchoscopy. In dose levels 3 (percutaneous) and 4 a volume of 10 ml of study medication was administered intratumorally by percutaneous injection under CT guidance. After treatment all patients were hospitalized in single rooms in an S2 isolation unit for at least 72 hr or until detection of adenovirus shedding by the patients became negative. All patients underwent a biopsy of the treated tumor lesion 24 to 48 hr after injection of SCH 58500. In dose levels, 1, 2, and 3 (endobronchial) posttreatment biopsies were obtained by bronchoscopy. In dose levels 3 (percutaneous) and 4 posttreatment biopsies were obtained by percutaneous needle biopsy under CT guidance.

Detection of wild-type p53 *gene transfer.* Expression of vector-specific wt *p53* RNA was assessed in posttreatment biopsies by means of RT-PCR according to previously published methods (Wills *et al.*, 1994). In brief, total cellular RNA was isolated from homogenized tumor biopsies and reverse transcribed into cDNA. The maximum amount of RNA available was used for RT-PCR. This ranged from 0.5 to 5 μg for samples from all patients except patient UPN 015, from whom 15 μg of RNA were obtained. PCR was performed for 28 cycles for all patient samples. Samples from patients treated at low dose levels with negative PCR results after 28 cycles were also run for 45 cycles in order to increase sensitivity. Those samples were from patients UPN 001, 002, 003, 004, 006, and 007, all of which remained negative after 45 PCR cycles. The vector-encoded *p53* cDNA was amplified using specific primers, one within the Ad2 tripartite leader (at the 5' end of the mRNA), and the second within the *p53* coding region, leading to amplification of a 563-bp target sequence. Parallel PCR reactions using primers specific for a 719-bp fragment of the human β-actin cDNA served as controls for integrity and efficacy of the RNA extraction as well as of the cDNA synthesis. Semiquantitation was carried out for vector-encoded *p53* cDNA as well as for human β-actin through the use of DNA "mimics" specific for each reaction. The mimic carried sequences at its ends that allowed it to be amplified by the same primers used to amplify the target sequence. This amplification results in a second band 412 bp in size for the "*p53* mimic," and 494 bp in size for the "β-actin mimic." Thus, on the gel, each lane should contain two bands: the upper band is the larger fragment target, and the lower band is the smaller fragment mimic. Addition of a known concentration of mimic to a given reaction enabled determination of the relative amount of each cDNA present. All PCR products and controls were run on agarose gels and stained with ethidium bromide. Quantification of the amplified DNA bands was performed on a Molecular Dynamics FluorImager (Molecular Dynamics, Sunnyvale, CA).

Virology studies. Adenovirus shedding and excretion were assessed in patient stool or rectal swab by means of a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Adenoclone; Cambridge Biotech, Worcester, MA) according to the manufacturer instructions. Further, adenovirus shedding in sputum and urine was assessed by the same ELISA kit according to an adapted protocol. In addition, an ELISA system was established to detect serum antibodies against SCH 58500, which also detects cross-reacting antibodies against wild-type adenoviruses. In brief, microtiter plates were coated overnight with SCH 58500, and then were washed and blocked for nonspecific binding with bovine serum albumin (BSA). Each serum sample was diluted 1:40 into phosphate-buffered saline (PBS) containing 1% BSA and 0.05% Tween, and then was serially diluted twofold. A total of seven dilutions of each serum sample was added to the plate and were incubated overnight. A positive control and pooled normal serum were also assayed on each plate. After washing, anti-SCH 58500 antibodies were detected by reaction with biotin-labeled protein A/G (Jackson ImmunoResearch Laboratories, West Grove, PA), horseradish peroxidase-labeled streptavidin, which resulted in a colorimetric reaction on the addition of hydrogen peroxide and tetramethyl benzidine. Samples were considered positive for the presence of anti-SCH 58500 antibodies if the ratio of the mean optical densities of the sample dilutions versus the mean optical densities of the normal human serum dilutions was above a threshold value of 0.28. Further, patients were considered positive for development of anti-SCH 58500 antibodies if the posttreatment sample was positive, and there was at least a twofold increase in mean optical density as compared with pretreatment values.

RESULTS

Wild-type p53 gene transfer

Fifteen patients were enrolled in the study between October 1, 1996, and July 16, 1997. Baseline characteristics are shown in Table 1. Nine patients were treated by bronchoscopic intratumoral injection of 1 ml of SCH 58500 at three different dose levels (levels 1, 2, and 3). Six patients were treated by percutaneous intratumoral injection of 10 ml of SCH 58500 at two different dose levels (levels 3 and 4). In 13 patients sufficient amounts of intact RNA were recovered from posttreatment biopsies for analysis of transgene expression by RT-PCR. The trial was closed on August 12, 1997. At dose level 1, no transgene expression could be detected, whereas at the subsequent dose levels expression of vector-specific wt *p53* RNA sequences (Fig. 1) could be demonstrated in a way suggestive of dose dependency. Results of wt *p53* RT-PCR in relation to SCH 58500 dose levels are summarized in Table 2. The median expression level of transgenic wt *p53* amounted to 8.01 molecules per 1000 molecules of β-actin (range, 0.49 to 197.97 molecules/1000 molecules β-actin); no correlation between transgene expression level and the vector dose injected could be established.

TABLE 1. PATIENT CHARACTERISTICS

Parameter	*Value/distribution*
n	15
Sex	4 female, 11 male
Age (median, range)	60 (45 to 75) years
Histology	
Adenocarcinoma	8
Squamous cell carcinoma	4
Large cell carcinoma	3
Pretreatment	
None/local procedures	6
Surgery	4
Radiotherapy	4
Chemotherapy	9
Active smokers	14

TABLE 2. SCH 58500 GENE TRANSFER BY DOSE

Dose level (PFU)	*Patients*	*Route*	*β-Actin*[a]	*p53*[b]
10^7	3	B[c]	3	0
10^8	3	B	3	1
10^9	3	B	2	2
	3	C[d]	3	2
10^{10}[e]	3 (+1)	C	2 (+1)	1 (+1)

[a] β-Actin, RT-PCR detection of human β-actin RNA as internal control.

[b] p53, RT-PCR detection of vector-specific wt *p53* sequences.

[c] B, Bronchoscopic intratumoral injection of 1 ml of SCH 58500 solution.

[d] C, CT-guided percutaneous intratumoral injection of 10 ml of SCH 58500 solution.

[e] At dose level 4, results of the second treatment of a patient initially treated at dose level 3 (percutaneous) are included in parentheses.

Clinical response and patient follow-up

Results of day 28 restaging CT scans in relation to wt *p53* gene transfer are summarized in Table 3. Whereas local disease stabilizations have been observed at treated tumor sites, in all but one evaluable patient distant tumor sites were progressive. According to standard oncological response criteria, at day 28 restaging 11 patients had progressive disease, 2 patients had stable disease, and 2 patients were not evaluable for tumor response. In one heavily pretreated patient, who was initially enrolled at dose level 3 (percutaneous), a stabilization of the treated chest wall metastasis was observed on day 28, whereas his untreated primary tumor progressed. Approximately 4 months after the first treatment, this patient received a second dose of SCH 58500 at dose level 4 on a single patient protocol exception after approval by the local ethics committee. The second injection was directed to the patient's primary tumor. Again, successful gene transfer was confirmed by RT-PCR from a biopsy taken 24 hr posttreatment (data included in Table 2). Approximately 8 hr posttreatment the patient experienced WHO grade 2 fever. No further adverse events were observed. On restaging on day 28 after the second dose, the primary tumor was again slightly progressive. On long-term follow-up, 9 of 15 patients died from progressive disease. Another patient died on day 18 of the observation period from progressive disease. As of December 1997, three patients are still alive with clinically stable disease, one patient is alive with slowly progressing disease, and one patient has been lost to follow-up. Clinical results of the six patients with successful demonstration of transgene expression in relation to SCH 58500 dose level are shown in Table 4.

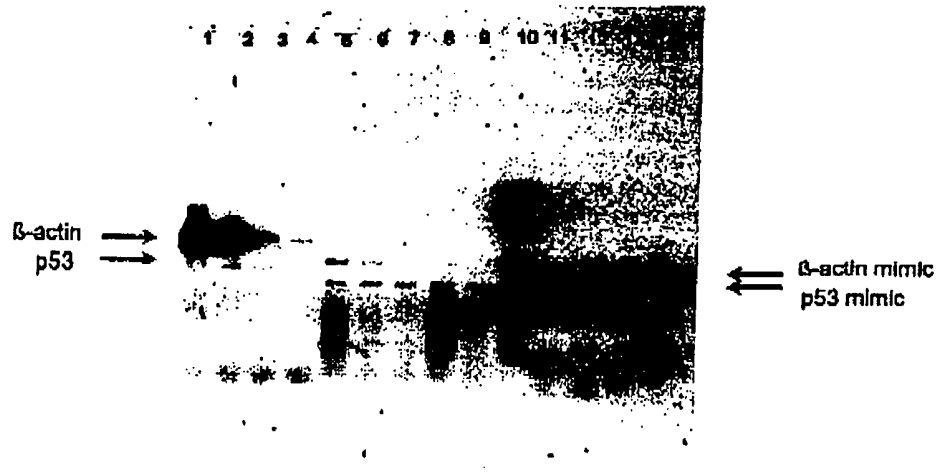

FIG. 1. Representative gel of an RT-PCR analysis of a patient sample. Four dilutions of the β-actin PCR product are shown in lanes 1–4, and four dilutions of the vector-specific *p53* PCR product are shown in lanes 5–8. A control to detect residual DNA is seen in lane 9, and DNA molecular weight marker is seen in lane 10. Low (500 molecules) and high concentrations (100,000 molecules) of the "mimics" for β-actin and vector-specific *p53* are seen in lanes 11 to 14.

TABLE 3. DISEASE STATUS ON DAY 28 IN RELATION TO WILD-TYPE *p53* TRANSGENE EXPRESSION

		Treated lesion			*Distant lesions*		
n	p53[a]	*PR*	*SD*	*PD*	*PR*	*SD*	*PD*
6	+	0	4	2	0	0	6
7[b]	−	0	2	3	0	1	3
2	ND	0	1	1	0	0	2

[a]p53, RT-PCR detection of vector-specific wt *p53* sequences.
[b]In two patients no day 28 CT scans were performed; in one patient no measurable distant disease was present.
Abbreviations: ND, Not done (insufficient RNA retrieved from posttreatment tumor biopsy); PR, partial remission; SD, stable disease; PD, progressive disease.

Toxicity

In general, toxicity of intratumoral injection of SCH 58500 was mild and mainly resulted from procedure-related discomfort. Nine patients experienced mild- to moderate-grade fever several hours after treatment, which spontaneously resolved no later than day 3. In addition, mild- to moderate-grade influenza-like symptoms, arthralgia, dyspnea, hypertension, and tachycardia were observed following SCH 58500 injection in one patient each. Adverse events that were considered to be related to the study treatment are summarized in Table 5. A transient increase in leukocyte counts was observed in eight patients without SCH 58500 dose dependency. There were five serious adverse events during the 28-day observation period: One patient treated at dose level 1 died on day 18 of the observation period. Postmortem examination revealed progressive disease and multiple pulmonary emboli as the cause of death. No signs of viral pneumonia were found, and no relation to study treatment could be established. Two patients experienced disease progression within the 28-day observation period. One patient experienced a hypertensive episode that required treatment. This patient had a past medical history of hypertension. One patient experienced diarrhea, malaise, and influenza-like symptoms, which required prolongation of his hospital stay. No adenovirus shedding was demonstrated in the sputum, urine, or stools of this patient, and the symptoms resolved on symptomatic treatment. There were no serious adverse events resulting from bronchoscopies, percutaneous injections, or biopsies.

Virology studies

In all patients adenovirus shedding was assessed on a daily basis by ELISA, performed on sputum, urine, stools, and rectal swabs before treatment and for 72 hr after treatment or until negative. All patients were confirmed to be negtive for adenovirus excretion before treatment. Posttreatment viral shedding was observed in the sputum of one patient only on day 2 after treatment at dose level 3 (percutaneous). In another patient treated at dose level 1 falsely positive adenovirus shedding in urine and stool was found until day 6, owing to inappropriate use of the ELISA kit. In all other patients no evidence of adenovirus shedding could be demonstrated.

In all patients anti-SCH 58500 antibodies (i.e., anti-adenoviral antibodies) were detectable before treatment. A significant (≥twofold) increase in anti-SCH 58500 antibodies was observed in 11 of 15 patients following treatment with SCH 58500. Relative antibody levels increased by day 7 and remained at that level until day 28 (Fig. 2). There was no correlation between relative antibody levels observed and the dose of SCH 58500 administered. In one patient receiving a second dose of SCH 58500, development of anti-SCH 58500 antibodies following the first treatment did not prevent a second successful local wt *p53* gene transfer (Fig. 3).

DISCUSSION

The most common human cancers are strongly selected for mutations of the tumor suppressor gene *p53*, underscoring its central role in prevention of malignant transformation and tumor progression. Moreover, mutant *p53* status appears to reduce sensitivity toward commonly applied cytotoxic agents and radiotherapy (Levine, 1997). Thus, transfer of wild-type *p53* is an attractive target for cancer gene therapy. Numerous *in vitro* studies have demonstrated growth inhibition (Takahashi *et al.*, 1992), restoration of chemotherapy sensitivity (Fujiwara *et al.*, 1994b), as well as induction of apoptosis (Fujiwara *et al.*, 1993)

TABLE 4. DISEASE STATUS AND LONG-TERM FOLLOW-UP IN RELATION TO SCH 58500 DOSE LEVEL OF SIX PATIENTS WITH SUCCESSFUL WILD-TYPE *p53* GENE TRANSFER

	Dose level		*Day 28*		
UPN	*(PFU)*	*Route*	*Treated*[a]	*Distant*[a]	*Follow-up*
005	10^8	B[b]	SD	PD	Died from PD
008	10^9	C[c]	PD	PD	Died from PD
009	10^9	C	SD	PD	Second treatment
010	10^9	B	SD[d]	PD	Died from PD
015	10^9	B	PD	PD	PD +2 months
012	10^{10}	C	SD	PD	SD +6 months

[a]Treated, treated tumor lesion; distant, untreated tumor lesion(s).
[b]B, Bronchoscopic intratumoral injection of 1 ml of SCH 58500 solution.
[c]C, CT-guided percutaneous intratumoral injection of 10 ml of SCH 58500 solution.
[d]Patient experienced local PR in treated tumor lesion at 2 months follow-up.
Abbreviation: UPN, Unique patient number.

TABLE 5. TREATMENT- AND/OR PROCEDURE-RELATED TOXICITIES IN RELATION TO SCH 58500 DOSE LEVEL[a]

	10^7 PFU (n = 3)[b]	10^8 PFU (n = 3)	10^9 PFU (n = 6)	10^{10} PFU (n = 3)
Hot flushes	II (1)	—	—	—
Flulike symptoms	—	—	II (1)	—
Arthralgia	—	—	—	I (1)
Fever	I (1)	I (2)	II (3)	II (3)
Dyspnea	—	—	II (1)	—
Hypertension	—	—	—	II (1)
Tachycardia	—	—	—	II (1)

[a]Roman numerals indicate maximal severity of adverse event (I, mild; II, moderate; III, severe; IV, life threatening). Digits in parentheses indicate number of patients experiencing the respective adverse event.
[b]*n*, Number of patients treated per dose level.

on wt *p53* gene transfer in lung cancer cells. Furthermore, in animal models of lung cancer a therapeutic effect of intratracheal instillation (Fujiwara *et al.*, 1994a) or intratumoral injection (Fujiwara *et al.*, 1994b; Wills *et al.*, 1994) of wt *p53*-containing expression vectors was noted. In these studies either retroviral (Cai *et al.*, 1993; Fujiwara *et al.*, 1993) or adenoviral (Zhang *et al.*, 1994; Harris *et al.*, 1996) vector constructs were applied with similar effects. An E1B-deficient adenoviral strain has been shown to replicate selectively in *p53* mutant tumor cells, leading to cytopathic effects at a low multiplicity of infection (Bischoff *et al.*, 1996; Heise *et al.*, 1997). This elegant approach used mutant *p53* as a target for virus-mediated tumor therapy without transferring wt *p53* itself.

An initial clinical pilot study reported induction of apoptosis and tumor regression in three of nine patients with advanced NSCLC treated by intratumoral injection of a fixed dose of a retroviral vector containing wt *p53* (Roth *et al.*, 1996). In that study vector DNA sequences were detected in some posttreatment tumor biopsies by DNA-PCR or by *in situ* hybridization. However, no transgene expression was demonstrated, limiting the interpretation of antitumoral and apoptotic effects observed in that trial.

In the present study intratumoral expression of vector-specific wt *p53* RNA as detected by RT-PCR was the primary end point. An adenoviral expression system was chosen because of its established safety in clinical trials, its organotropism, and its ability to transduce noncycling as well as dividing tumor cells (Zabner *et al.*, 1993; Crystal *et al.*, 1994, 1995; Hay *et al.*, 1995; Knowles *et al.*, 1995). In addition, the antitumoral efficacy of adenoviral gene transfer of wt *p53* has been demonstrated in several *in vitro* and preclinical *in vivo* models (Wills *et al.*, 1994; Harris *et al.*, 1996). Intratumoral transgene expression could be demonstrated in a manner suggestive of dose dependency in tumor samples taken within 48 hr posttreatment. Virus doses of 10^9 PFU were required to achieve detectable transgene expression in the majority of cases. The toxicity of the treatment in general was mild to moderate. Febrile reactions tended to increase with the vector dose administered. However, this inflammatory response did not prevent local wt *p53* gene transfer at higher virus doses. Moreover, evidence suggestive of short-term local tumor control was obtained. To minimize the risk for the patients only small posttreatment tumor samples could be obtained. Thus, the functional activity of the transgene in terms of induction of proteins encoded by genes downstream of *p53* could not be assessed. Antitumoral effects could therefore also be mediated by the adenovirus itself (Bischoff *et al.*, 1996) or by an anti-adenoviral immune reaction. In addition, an immune reaction against p53 peptides presented by tumor cells (Theobald *et al.*, 1995; Vierboom *et al.*, 1997) could have been augmented by intratumoral injection of SCH 58500.

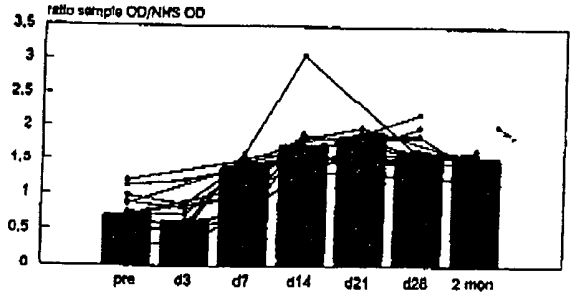

FIG. 2. Development of anti-SCH 58500 antibodies in relation to treatment. The boxes show the median values at each time point. OD, Optical density; NHS, normal human serum.

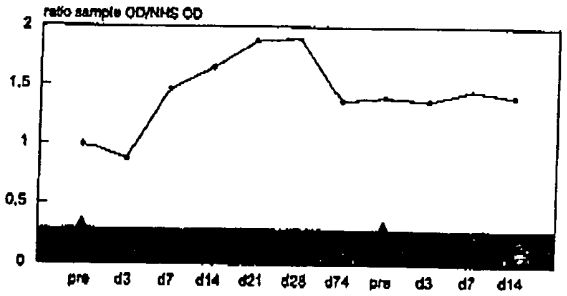

FIG. 3. Course of anti-SCH 58500 antibodies in a single patient receiving two dosings of SCH 58500. The black triangles show the time points of treatment. The shaded area highlights the negative threshold of 0.28.

Clinical effects, such as local disease stabilization, as observed in this phase I study should be interpreted with caution. More importantly, intratumoral injection of SCH 58500 even at high viral doses was shown to be safe and feasible in patients with advanced NSCLC. In conclusion, for the first time intratumoral transgene expression of wt *p53*, as obtained by adenovirus-mediated gene transfer, could be demonstrated in a clinical phase I study. An ongoing phase II study focuses on clinical efficacy of repetitive injections of SCH 58500 in conjunction with systemic cytotoxic chemotherapy.

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